AD NUMBER

AD422367

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Sep 1963. Other requests shall be referred to U.S. Army Biological Labs., Frederick, MD.

AUTHORITY

BORL D/A ltr, 27 Sep 1971

TECHNICAL MANUSCRIPT 96

SIMPLE AND SENSITIVE METHOD FOR DEMONSTRATING MERCAPTAN PRODUCTION BY MICROORGANISMS

SEPTEMBER 1963

UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 96

A SIMPLE AND SENSITIVE METHOD FOR DEMONSTRATING MERCAPTAN PRODUCTION BY MICROORGANISMS

Warren P. Iverson

Special Operations Division
DIRECTOR OF DEVELOPMENT

Project 1C54-360-3D419

September 1963

Expenditure orders were 2059 and 5240.
This material was originally submitted
as manuscript 5209.

SIMPLE AND SENSITIVE METHOD FOR DEMONSTRATING MERCAPTAN PRODUCTION BY MICROORGANISMS

ABSTRACT

A simple and sensitive test for demonstrating mercaptan formation by microorganisms has been described. A positive test for mercaptans is indicated by the formation of white vapors when a few drops of iodine-azide reagent (3 grams of sodium azide in 100 milliliters of 0.1N iodine) is added to one or two milliliters of a broth culture. A positive reaction has been observed with several species of Pseudomonas, a species of Serratia, and a few lactose-fermenting species.

There is very little information in the literature concerning the production of mercaptans by microorganisms. Mercaptan production has been reported for anaerobes,[1] fungi,[2] Escherichia coli,[3] and Proteus vulgaris.[4] Conversely, Masatashi[4] and Almy and James[5] were not able to demonstrate any mercaptan production by E. coli, nor were Almy and James able to demonstrate any production by P. vulgaris. The production of mercaptans must be widely distributed among bacteria, however, since the terms "foul-smelling," "putrid," etc., are commonly found in the literature.

Feigl[6] mentions two tests, (a) the catalytic acceleration of the iodine-azide reaction and (b) the precipitation of cuprous salts, which may be used to detect thioketones and mercaptans. While testing a number of bacterial isolates by the iodine-azide reaction, it was noted with several of the Pseudomonas, Serratia, and lactose-fermenting species that white vapors were produced when two drops of the iodine-azide reagent (three grams of sodium azide in 100 milliliters 0.1N iodine) were added to one or two milliliters of inoculated one per cent Trypticase (Baltimore Biological Laboratories) broth. This was not expected, since Feigl[6] states that a positive reaction for mercaptans is characterized by the "evolution of little bubbles of nitrogen." Slight gas evolution was, however, noted in almost all the cultures upon the addition of the reagent.

In verifying this reaction further, a similar production of white vapor was noted when a loopful of the iodine-azide reagent was held near the following pure compounds: n-amyl-, n-hexyl-, tert-hexyl-, n-octyl-, tert-octyl-, and n-decyl-mercaptan. When a loopful of the reagent was held in a stream of hydrogen sulfide, only bubbles were produced. It thus appears that white vapor formation rather than bubble formation is an indication of mercaptan compounds.

White vapors were noted in the case of amyl mercaptan at a dilution of 1:1,000,000 (one drop of the reagent added to one milliliter of distilled water containing 0.85 microgram of amyl mercaptan). The limit of sensitivity appeared to be between 0.85 and 0.45 microgram per milliliter.

For best observation of the vapors, it is recommended that the tube be held in front of a bright light, preferably a microscope lamp. It is also suggested that screw-cap tubes be used and that an additional source of organic sulfur be added (viz., cystine, 0.1 per cent) to the medium.

The nature of this reaction is being investigated further, and it has proved most useful in the classification of a number of Pseudomonas species. This reaction appears to hold further promise as a taxonomic tool for a great variety of other microorganisms.

LITERATURE CITED

1. Rettger, L.F. "Studies on putrefaction," J. Biol. Chem. 2:71-86, 1906.

2. Challenger, F. "Review of biological methylation," Sci. Prog. 35:396-416, 1947.

3. Fromageot, C., and Moubacher, R. "Enzymic production of hydrogen sulfide by sulfur-containing organic compounds," Enzymologia 2:121-128, 1937.

4. Masatashi, K. "The production of mercaptans from l-cystine by bacteria," Biochem. Z. 136:198-202, 1923.

5. Almy, L.H., and James, L.H. "Formation of sulfur compounds by bacteria," J. Bacteriol. 12:319-331, 1926.

6. Feigl, F. "Spot tests," Vol. 2, "Organic applications," New York, American Elsevier Publishing Co., 1954. pp. 164-168.